CLAIMS

We claim.

1. A method of detecting an activity in a cell, comprising:

1) providing a cell comprising,

a) at least one destabilization domain, wherein said destabilization domain is non-cleavable by α-NH-ubiquitin protein endoproteases,

b) a reporter moiety, and

c) a linker moiety that operatively couples said destabilization domain to said reporter moiety,

wherein said linker moiety comprises a recognition motif for said activity and modification of said linker moiety by said activity modulates the coupling of said destabilization domain to said reporter moiety thereby modulating the stability of said reporter moiety, and

wherein said linker moiety is non-cleavable by said α-NH-ubiquitin protein endoproteases,

2) detecting said reporter moiety, or a product of said reporter moiety.

2. The method of claim 1, wherein said at least one destabilization domain is arranged as linear multimer, and

wherein said linear multimer comprises at least two copies of said destabilization domain and is non-cleavable by said α-NH-ubiquitin protein endoproteases.

3. The method of claim 1, wherein said linker moiety is non-naturally occurring polypeptide or protein.

4. The method of claim 1, wherein said linker moiety covalently couples said destabilization domain to said reporter protein.

5. The method of claim 1, wherein said linker moiety is between about 1 and 30 amino acid residues.

6. The method of claim 1, wherein said destabilization domain comprises a ubiquitin homolog.

7. The method of claim 6, wherein said ubiquitin homolog comprises a mutation that prevents cleavage by said α-NH-ubiquitin protein endoproteases.

8. The method of claim 6, wherein said ubiquitin homolog comprises a mutation at glycine 76.

9. The method of claim 1, wherein said linker moiety comprises a first amino acid sequence that is covalently coupled to said reporter moiety, and a second amino acid sequence that is covalently coupled to said at least one destabilization domain.

10. The method of claim 1, wherein said activity is selected from the group consisting of a protease activity, a protein kinase activity and a phosphoprotein phosphatase activity.

11. The method of claim 1, wherein said reporter moiety is selected from the group consisting of a naturally fluorescent protein homolog, a β-lactamase homolog, a β-galactosidase homolog, an alkaline phosphatase homolog, a CAT homolog, and a luciferase homolog.

12. The method of claim 11, wherein said reporter moiety comprises a β-lactamase homolog.

13. The method of claim 11, wherein said reporter moiety comprises an *Aequorea* Green fluorescent protein homolog.

14. The method of claim 11, wherein said reporter moiety comprises an Anthozoan Green fluorescent protein homolog.

15. The method of claim 1, wherein said cell is a mammalian cell.

16. The method of claim 1, wherein said cell is a yeast cell.

17. The method of claim 1, wherein said cell is an insect cell.

18. The method of claim 1, wherein said cell is a plant cell.

19. The method of claim 1, wherein said method further comprises the step of adding a protein synthesis inhibitor to said cell.

20. The method of claim 1, wherein said method further comprises the step of adding an inhibitor of said reporter moiety to said cell.

21. The method of claim 1, wherein said method further comprises the step of adding a test chemical to said cell.

22. The method of claim 20, wherein said method further comprises the step of relating said reporter moiety activity before addition of said test chemical to said reporter moiety activity after addition of said test chemical.

23. A method of regulating the concentration of one or more target proteins in a cell, comprising;

1) providing a cell comprising,

a) a linear multimerized destabilization domain, wherein said linear multimerized destabilization domain is non-cleavable by a α-NH-ubiquitin protein endoproteases, and comprises at least two copies of a destabilization domain,

b) a target protein, and

c) a linker that operatively couples said linear multimerized destabilization domain to said target protein,

wherein said linker comprises a protease cleavage site for a protease and cleavage of said linker by said protease modulates the coupling of said linear multimerized destabilization domain to said target protein, thereby modulating the stability of said target protein in said cell, and

wherein said linker is non-cleavable by a α-NH-ubiquitin protein endoproteases,

2) providing said protease to cause cleavage of said linker thereby increasing the stability and concentration of said protein of interest in said cell.

24. The method of claim 23, wherein said protease is naturally expressed in said cell.

25. The method of claim 23, wherein said protease is not naturally expressed in said cell.

26. The method of claim 23, further comprising the step of adding an inhibitor of said protease.

27. The method of claim 23, wherein said linker is between 1 and 30 amino acid residues.

28. The method of claim 23, wherein said cell is a mammalian cell.

29. The method of claim 23, wherein said cell is a yeast cell.

30. The method of claim 23, wherein said cell is an insect cell.

31. The method of claim 23, wherein said destabilization domain comprises a ubiquitin homolog.

32. The method of claim 31, wherein said ubiquitin homolog comprises a mutation that prevents cleavage by α-NH-ubiquitin protein endoproteases.

33. The method of claim 31, wherein said ubiquitin homolog comprises a mutation at glycine 76.

34. The method of claim 23, wherein said protease is provided by transfecting said cell with an expression vector comprising a nucleic acid sequence encoding said protease.

35. The method of claim 34, wherein said expression vector further comprises an inducible promoter.

36. The method of claim 34, wherein said expression vector is a retroviral expression vector.

37. The method of claim 34, wherein said protease is a viral protease.

38. A method of destabilizing a target protein in a cell, comprising;

operatively coupling a target protein to a linear multimerized destabilization domain, wherein said linear multimerized destabilization domain is non-cleavable by a α-NH-ubiquitin protein endoproteases, and comprises at least two copies of a destabilization domain.

39. The method of claim 38, wherein said destabilization domain comprises a ubiquitin homolog.

40. The method of claim 39, wherein said ubiquitin homolog comprises a mutation that prevents cleavage by α-NH-ubiquitin protein endoproteases.

41. The method of claim 39, wherein said ubiquitin homolog comprises a mutation at glycine 76.

42. The method of claim 38, wherein said protein of interest is fused in frame to said multimerized destabilization domain.

43. The method of claim 38, wherein said protein of interest is non-covalently coupled to said multimerized ubiquitin fusion protein.

44. The method of claim 38, wherein said cell is a mammalian cell.

45. The method of claim 38, wherein said cell is a yeast cell.

46. The method of claim 38, wherein said cell is an insect cell.

47. The method of claim 38, wherein said cell is a plant cell.

48. The method of claim 38, wherein said target protein is coupled to said multimerized destabilization domain by a linker.

49. The method of claim 48, wherein said linker is between 1 and 10 amino acid residues.

50. A recombinant DNA molecule, comprising a nucleic acid sequence encoding for;

a) a linear multimerized destabilization domain, wherein said linear multimerized destabilization domain is non-cleavable by a α-NH-ubiquitin protein endoproteases, and comprises at least two copies of a destabilization domain,

b) a target protein, and

c) a linker moiety that operatively couples said multimerized destabilization domain to said reporter moiety,

wherein said linker is non-cleavable by a α-NH-ubiquitin protein endoproteases.

51. The method of claim 50, wherein said linker moiety comprises an enzyme modification site for an activity, and modification of said linker moiety by said activity modulates the coupling of said multimerized destabilization domain to said reporter moiety, thereby modulating the stability of said reporter moiety.

52. The method of claim 50, wherein said destabilization domain comprises a ubiquitin homolog.

53. The method of claim 52, wherein said ubiquitin homolog comprises a mutation that prevents cleavage by α-NH-ubiquitin protein endoproteases.

54. The method of claim 52, wherein said ubiquitin homolog comprises a mutation at glycine 76.

55. A recombinant protein molecule, comprising an amino acid sequence encoding for;

a) a linear multimerized destabilization domain, wherein said multimerized destabilization domain is non-cleavable by a α-NH-ubiquitin protein endoproteases, and comprises at least two copies of said destabilization domain,

b) a target protein, and

c) a linker moiety that operatively couples said multimerized destabilization domain to said reporter moiety,

wherein said linker is non-cleavable by a α-NH-ubiquitin protein endoproteases.

56. The method of claim 55, wherein said linker moiety comprises a recognition motif for an activity, and modification of said linker moiety by said activity modulates the coupling of said multimerized destabilization domain to said reporter moiety, thereby modulating the stability of said reporter moiety.

57. The method of claim 55, wherein said destabilization domain comprises a ubiquitin homolog.

58. The method of claim 57, wherein said ubiquitin homolog comprises a mutation that prevents cleavage by α-NH-ubiquitin protein endoproteases.

59. The method of claim 57, wherein said ubiquitin homolog comprises a mutation at glycine 76.

60. A host cell, comprising a nucleic acid sequence encoding for;

a) a linear multimerized destabilization domain, wherein said multimerized destabilization domain is non-cleavable by a α-NH-ubiquitin protein endoproteases, and comprises at least two copies of said destabilization domain,

b) a target protein, and

c) a linker moiety that operatively couples said multimerized destabilization domain to said reporter moiety,

wherein said linker is non-cleavable by a α-NH-ubiquitin protein endoproteases.

61. The method of claim 60, wherein said destabilization domain comprises a ubiquitin homolog.

62. The method of claim 61, wherein said ubiquitin homolog comprises a mutation that prevents cleavage by α-NH-ubiquitin protein endoproteases.

63. The method of claim 61, wherein said ubiquitin homolog comprises a mutation at glycine 76.

64. A transgenic animal, comprising a nucleic acid sequence encoding for;

d) a linear multimerized destabilization domain, wherein said multimerized destabilization domain is non-cleavable by a

α-NH-ubiquitin protein endoproteases, and comprises at least two copies of said destabilization domain,

e) a target protein, and

f) a linker moiety that operatively couples said multimerized destabilization domain to said reporter moiety,

wherein said linker is non-cleavable by a α-NH-ubiquitin protein endoproteases.

65. The method of claim 64, wherein said destabilization domain comprises a ubiquitin homolog.

66. The method of claim 65, wherein said ubiquitin homolog comprises a mutation that prevents cleavage by α-NH-ubiquitin protein endoproteases.

67. The method of claim 65, wherein said ubiquitin homolog comprises a mutation at glycine 76.

68. A transgenic plant, comprising a nucleic acid sequence encoding for;

a) a linear multimerized destabilization domain, wherein said multimerized destabilization domain is non-cleavable by a α-NH-ubiquitin protein endoproteases, and comprises at least two copies of said destabilization domain,

b) a target protein, and

c) a linker moiety that operatively couples said multimerized destabilization domain to said reporter moiety,

wherein said linker is non-cleavable by a α-NH-ubiquitin protein endoproteases.

69. The method of claim 68, wherein said destabilization domain comprises a ubiquitin homolog.

70. The method of claim 69, wherein said ubiquitin homolog comprises a mutation that prevents cleavage by α-NH-ubiquitin protein endoproteases.

71. The method of claim 69, wherein said ubiquitin homolog comprises a mutation at glycine 76.

72. A method for identifying a modulator of an activity, comprising;

a) contacting a cell with a test chemical, wherein said cell comprises,

i) at least one destabilization domain, wherein said destabilization domain is non-cleavable by α-NH-ubiquitin protein endoproteases,

ii) a reporter moiety, and

iii) a linker moiety that operatively couples said destabilization domain to said reporter moiety,

wherein said linker moiety comprises a recognition motif for said activity and modification of said linker moiety by said activity modulates the coupling of said destabilization domain to said reporter moiety thereby modulating the stability of said reporter moiety, and

wherein said linker moiety is non-cleavable by said α-NH-ubiquitin protein endoproteases,

b) detecting said reporter moiety, or a product of said reporter moiety in the presence of said test chemical, and

c) comparing said reporter moiety activity from step b) to the reporter moiety activity in a control cell in the absence of said test chemical.

73. The method of claim 72, further comprising the step of contacting said cell with an activator of said activity prior to the addition said test chemical.

74. The method of claim 72, further comprising the step of detecting the viability of said cell

75. The method of claim 72, wherein said activity is selected from the group consisting of a protease activity, a protein kinase activity and a phosphoprotein phosphatase activity.

76. The method of claim 72, wherein said reporter moiety is selected from the group consisting of a naturally fluorescent protein homolog, a β-lactamase homolog, a β-galactosidase homolog, an alkaline phosphatase homolog, a CAT homolog, and a luciferase homolog.

77. A test chemical identified by any of the methods of claims 72, 73, 74, 75 or 76.

78. A pharmaceutical composition comprising a test chemical identified by any one of the methods of claims 72, 73, 74, 75 or 76.

79. The pharmaceutical composition of claim 78, further comprising a pharaceutically acceptable carrier.

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